THE IDENTIFICATION AND COMPARISON OF THE FLAVONOIDS IN THE FLOWERS OF CAMPANULA RAPUNCULOIDES L. AND CAMPANULA AMERICANA L.

An abstract of a Thesis by
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July 1975
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The problem. The purpose of this study was to identify and compare the flavonoids found in the flowers of Campanula rapunculoides L. and C. americana L.

Procedure. Flavonoids were extracted in 1% HCl-methanol solution and concentrated on a rotary vacuum evaporator. Filter paper chromatography was used to separate and purify the pigments. Both crude and purified pigments were hydrolyzed and tests run to gain information about the aglycones and attached sugars. A spectrophotometer was used to record the absorption spectra of purified pigments.

Findings. Two anthocyanins which appear to be glycosides of delphinidin and another flavonoid which may be a 7,8-dihydroxyflavanone were found in both C. rapunculoides and C. americana. Vitexin and luteolin were found in C. americana but not in C. rapunculoides. C. rapunculoides contains an unidentified, blue florescing flavonoid not found in C. americana.

Conclusions. It was shown that C. rapunculoides and C. americana share three flavonoids while there are two in C. americana and one in C. rapunculoides which are not shared.

Recommendations. Further work is needed to isolate and identify the sugar attachments on the flavonoids. Other species of Campanula need to be examined to show their relationship to C. americana and C. rapunculoides.

THE IDENTIFICATION AND COMPARISON OF THE FLAVONOIDS IN THE FLOWERS OF CAMPANULA RAPUNCULOIDES L. AND CAMPANULA AMERICANA L.

A Thesis

Presented to

The School of Graduate Studies

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

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by

Walter Eugene Buckles

July 1975

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INTRODUCTION AND REVIEW OF LITERATURE

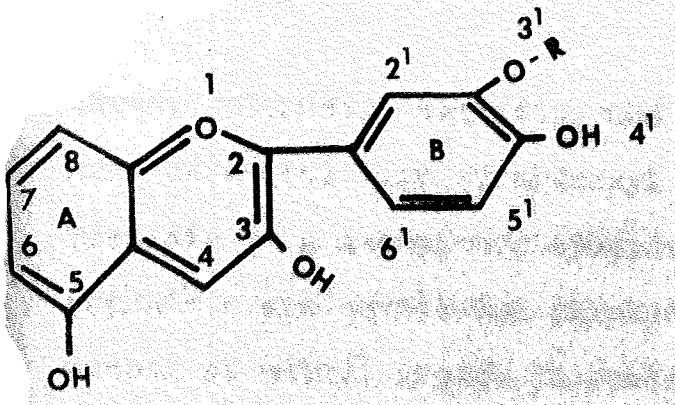
Pigmentation of plants is an obvious and recognizable fact. The chlorophyll enables the plants to collect energy for photosynthesis. However, the flowers are structures which are most varied in their pigmentation and present a beautiful array of colors to attract insect pollinators and man's asthetic attention. Flower pigments are divided into two broad groups (Clevenger, 1964; Arditti and Dunn, 1969). Fat soluble carotenoids found in the plastids are hues of red, orange, and yellow. Flavonoids are water soluble and found in cell vacuoles. They provide red, blue, and yellow shades in the flower colors. The purpose of this study was to identify and compare the flavonoid pigments in the flowers of Campanula rapunculoides L. and Campanula americana L.

Flavonoid pigments are divided into four general groups (Harborne, 1967). These are: (1) anthocyanins; (2) flavones; (3) chalcones and aurones; and (4) flavanones and isoflavones. Anthocyanins and flavones make up the major flavonoids of plants (Harborne, 1967) being very numerous and widely distributed in nature. Chalcones and aurones, and flavanones and isoflavones, along with some rarer forms, the biflavonyls and leucoanthocyanidins, are considered to be minor flavonoids (Harborne, 1967). The basic chemical structure of all flavonoids consists of two six-carbon-atom benzine rings linked together by a chain of three carbon atoms

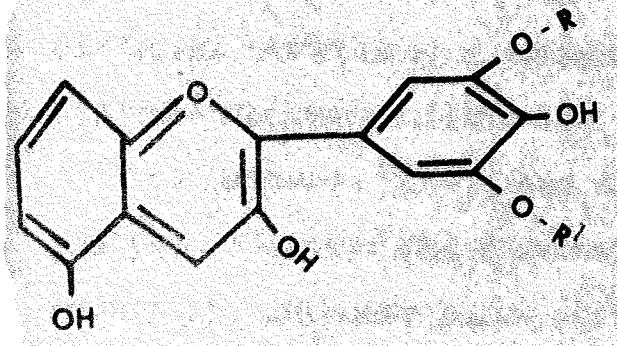
(Clevenger, 1964).

According to Harborne (1967) anthocyanins are responsible for nearly all of the pink, scarlet, red, mauve, violet, and blue colors in the petals and leaves of higher plants. All anthocyanins are based chemically on the cyanidin molecule (Figure 1). In nature the pigments usually occur as anthocyanins. These are the anthocyanidin glycosides containing one or more sugar molecules and sometimes acylated with organic acid residues (Hayashi, 1962; Harborne, 1958a). When the sugars are attached the pigment is called an anthocyanin. The pigment without the sugars is called an anthocyanidin (Clevenger, 1964). Harborne (1967) refers to the molecule with no attachments as an aglycone. The positions at which the sugars may be bound to the anthocyanidin usually are restricted to the 3-position or the 3- and 5-positions (Harborne, 1958a).

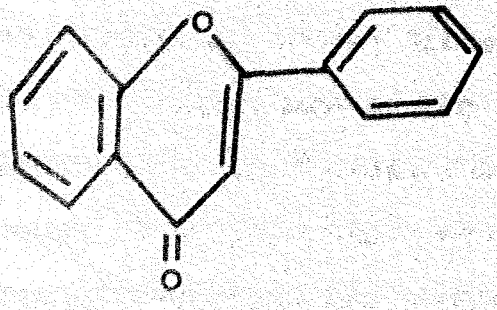
According to Clevenger (1964) anthocyanidins differ from each other in the number and arrangement of chemical groups bound to the B ring of the molecule. Six common anthocyanidins have been identified and their structures determined (Clevenger, 1964; Harborne, 1967). These are cyanidin, pelargonidin, delphinidin, peonidin, petunidin, and malvidin. Three of these, cyanidin, pelargonidin, and delphinidin, differ from one another by the number of hydroxyl groups found on the B ring. As the number of hydroxyl groups increases, the blueness of the flower deepens. The other



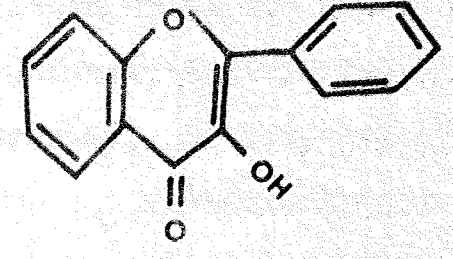
cyanidin R=H
peonidin R=Me
pelargonidin OR=H



delphinidin R=R¹=H
petunidin R=Me
R¹=H
malvidin R¹=R=Me



Flavone



Flavonol

Figure 1. Basic structures of flavonoid skeletons.

three, petunidin, malvidin, and peonidin, differ in the degree of methylation of hydroxyl groups present on the B ring. Peonidin is a methylated cyanidin while malvidin and petunidin are methylated delphinidins. An increase in the number of methyl groups increases the redness of the flowers. Thus the wide range of anthocyanin colors is possible through the addition of various sugar and organic acid molecules at different sites and in different combinations to each of the different anthocyanidins.

Harborne (1967) has divided the flavones in two groups, flavones and flavonols. These are the most abundant groups of compounds based on the flavonoid skeleton. They are more highly oxidized than the anthocyanins and provide a range of yellow colors. The structures of the parent compounds are given in Figure 1. The addition of a hydroxyl group at the 3-position on the flavonol is the only difference between the basic structure of the two groups. Gripenberg (1962) placed both the flavone and flavonals together in the flavone group. But since there are between two and three hundred known flavone and flavonol compounds a division is helpful. Within these two groups the main structural variations are confined to differences in the number and position of the hydroxyl, methyl, and glycosyl attachments (Harborne, 1959).

Harborne (1967) lists two common flavones, apigenin and luteolin, and thirty-four rarer flavones. As in the anthocyanins the flavone aglycones may be altered in their

properties by the addition of various chemical groups. There are three common flavonols; kaempferol, quercetin, and myricetin, along with several rarer structures formed by hydroxylation and methylation. These in turn may form glycosidic hookups thus providing a wide variety of compounds. Hattori (1962) has written a review of the glycosides of the flavones and flavonols.

Classical studies by Robinson and Robinson (1934) enabled them to characterize the anthocyanins in over 139 different plants. Bate-Smith (1948) developed a method of quickly detecting flavonoid pigments using filter paper chromatography. He found that 1% hydrochloric acid worked well for extracting the pigments and had several advantages in keeping the spots compact on the paper. The n-butanol-acetic acid-water (40:10:50) solvent that he devised is now extensively used in pigment chromatography. He also found that the spots on the paper after developing had distinctive colors both in ordinary and ultraviolet light and that the colors changed in a distinctive way when treated with ammonia vapors. The distance a spot travels on the paper divided by the distance the solvent front travels gives the R_f value for that spot (Harborne, 1958a; Mabry et al., 1970). This value varies with the type of solvent and structure of the flavonoid and so is a valuable tool for identification purposes.

Many different extracting solutions, developing

solvents, and procedures have been tried with the filter-paper-chromatography method of pigment detection. There seems to be as much variety in methods and materials as there are those who have worked in this area. Francis (1967) lists a summary of five steps necessary for the identification of anthocyanins. Gage and his colleagues (1951) showed the chromatographic behavior of thirty-eight flavonoid-type compounds in eleven different solvent systems and observed the colors in visible and ultraviolet light along with various reagents sprayed on the spots to give color changes due to chemical reactions.

Separation of the pigments using thin layer chromatography plates has been developed as well as column chromatography and electrophoresis. Many of the specific chromatographic and electrophoretic techniques are described by Smith (1969) and a general review of these methods is presented by Seikel (1962). Thompson (1959) presented an entire issue of the Botanical Review on partition chromatography. Gupta (1968) developed a sensitive thin layer chromatography technique to quickly examine and establish the number of anthocyanins and their aglycones.

McClure and Alston (1966) and Harborne (1958a) obtained purified specific flavonoids by streaking crude extracts across the bottom of Whatman No. 3 filter paper and developing in tertiary butanol:acetic acid:water (3:1:1) or acetic acid:water (15:85) to separate the pigment into

distinctive bands. These bands were then cut out of the paper and the pigments eluted from the paper in 0.01% HCl in methanol. Many similar methods with different solvents and eluting agents have been used.

Harborne (1958b) used spectrophotometry to characterize anthocyanins. He found that each anthocyanin had a maximum spectral absorption at a distinctive region of the absorption spectrum. Reagents such as aluminum chloride which produce shifts in the spectral maxima in accordance with the location of the functional groups on the flavonoid molecule have greatly increased the value of spectral data in flavonoid identification and structural analysis (Jurd, 1962). Pollock and his colleagues (1967) found that the shifts of the absorption peak in the visible range toward longer wavelengths (bathochromic) are brought about by the aluminum ions chelating with ortho-dihydroxy groupings. These shifts occur with cyanidin, delphinidin, and petunidin, but not other common anthocyanidins. Flavones and flavonols which contain hydroxyl groups at the 3- or 5-positions form acid stable complexes which cause a bathochromic shift in the spectral maxima and aluminum chloride plus hydrochloric acid causes spectral shifts in 3,5-dihydroxyflavones (Mabry et al., 1970). Stewart et al. (1969) used spectrophotometry to measure the optical density in intact tissues of roses, poinsettas, and snapdragons and correlated this to the optical density of anthocyanins in extracts.

Aglycones can be obtained by acid hydrolysis of the naturally occurring glycosides and there are many methods used (Harborne, 1967). Both the sugars and aglycones from hydrolysis can then be identified by chromatographic means. Abe and Hayashi (1956) used a solution of a glycoside in 1% hydrochloric acid in methanol mixed with an equal volume of 20% aqueous hydrochloric acid and heated in a water bath at 70° C. During the hydrolysis samples were removed at regular intervals and spotted on filter paper and developed. Their experiments showed that hydrolysis proceeded slowly and that diglycosides were degraded stepwise into monoglycosides. Halery and Asen (1959) used equal parts of concentrated purified pigment and 2N hydrochloric acid at 100° C for forty-five minutes under reflux and removed the aglycones with a small amount of iso-amyl alcohol. Fahselt and Ownby (1968) used a methanol and hydrochloric acid solution and heated the pigments in a boiling water bath for ten to fifteen minutes. Muszynski (1964) found malvidin and petunidin in blue and purple petunias and cyanidin and peonidin in red varieties of acid hydrolysis of the naturally occurring anthocyanins. Pollock et al. (1967) followed similar procedures for hydrolysis except they boiled in the dark for thirty minutes. The sugars produced by acid hydrolysis are identified by paper chromatography (Harborne, 1967). By spotting the sugar residues along with known sugars and developing in several solvent systems these can be identified.

Bate-Smith (1963) reported the exceptional usefulness of the flavonoid constituents as taxonomic guides is due to the fact that they are not actually concerned in cellular metabolic processes. He said that any particular flavonoid constituent can be relied on to be present in more or less constant amounts in the same tissue of the same species so long as the plants are grown under normal physiologically healthy conditions. Chemical plant taxonomy is a topic which has developed rapidly since 1957 (Harborne, 1967). Alston et al. (1962) used chromatographic analysis of leaf extracts to show the relationship and inheritance patterns of certain components in Baptisia, and Torres and Levin (1964) used chromatography to get information leading to the ancestry of Zinnia taxa. Genes controlling the hydroxylation of cyanidin to delphinidin were reported by Dedio et al. (1969) to have been uncovered in many plants. Brown and his colleagues (1969) found that chromatographic flavonoid patterns for individual species are usable in identification and can be used to determine relationships that are not otherwise noted or to verify relationships about which there is doubt when species are classed morphologically. Since a given pigment is synthesized by enzymes which are genetically determined, then similar compounds are present due to genetic similarities and should show some degree of closeness of relationship between populations (personal communication with S. Lane Wilson, Department of Biology, Drake University,

Des Moines, Iowa). Arditti (1969) used chromatography and spectrophotometric methods to identify floral anthocyanins of several species of orchids. His findings suggested that several were separate species and that two should not be combined. The study of flavonoids from leaves of twelve species of Linaria proved to be useful for solving taxonomic problems (Valdes, 1970). Noriyuki (1971) found the presence of rare flavonoids and of structural modifications in groups of plants are useful as chemical markers in classification and phylogeny. Durkee and Harborne (1973) found that it was possibly the glycosidic patterns and not the aglycones which are of taxonomic value between species and genera in the Cruciferae.

Little work has been done with Campanula and related genera. Harborne (1967) examined one species of Campanula and found a 3,5-diglucoside with delphinidin as the aglycone. Wilson (1968) compared two species of Lobelia and found pelargo-nidin-3-monoglucoside as the main pigment in one and a delphinidin-3,5-diglucoside in the other. The relationship between Campanula and Lobelia is not clear. Deam (1940) listed them as separate families in the order Campanulales while Gray (1950) and Rendle (1925) list them each as sub-families in the family Campanulaceae. Bell (1967) however includes both as genera in that family. Conrad (1948, 1951) lists them as separate families in his 6th edition but has them as separate genera in the same family in his 7th edition.

MATERIALS AND METHODS

The Campanula rapunculoides L. and C. americana L. used in this study were identified according to Gleason (1968). The flowers were collected from naturally growing plants in Polk and Warren counties in Iowa during the summer of 1974.

The petals were placed in 1% HCl-methanol solution and kept at 4° C overnight to extract the pigments. This crude extract was concentrated by evaporation between 30° C and 40° C on a rotary vacuum evaporator (Rinco Model 305137).

Two dimensional filter paper chromatography was used for the extract of each species to get an idea of the pigments present. The concentrated crude extracts were spotted in the lower right hand corners of Whatman No. 1 and Whatman No. 3 filter papers. Two different developing solvent systems were used. Some were developed using solvent a (Table 1) for the first direction and solvent c (Table 1) for the second direction. Others were developed using solvents i and j (Table 1) for the first and second directions respectively. The chromatograms were air dried and the spots marked on each paper for each solvent. They were observed in both visible and ultraviolet light before and after exposure to ammonia fumes.

Table 1. Chromatographic solvents

Abbreviation	Composition	Volume Ratio
a BAW	n-butanol:acetic acid:water	4:1:5*
b 1% HCl	conc. hydrochloric acid:water	3:97
c HoAcHCl	acetic acid:conc. hydrochloric acid:water	15:3:82
d BuHCl	n-butanol:2Nhydrochloric acid	1:1**
e Formic	acetic acid:conc. hydrochloric acid:water	30:3:10
f Forrestal	formic acid:conc. hydrochloric acid:water	5:2:3
g BEW	n-butanol:ethanol:water	40:11:10
h EaPyW	ethyl acetate:pyridine:water	8:2:1
i TBA	tertiary butanol:acetic acid:water	3:1:1
j 15% HoAc	acetic acid:water	15:85
k H_2O	distilled water	

*top (organic) layer used
**paper equlbrated in lower aqueous phase of BuHCl for 24 hours

To obtain purified pigments a 1 cm band of the crude extract was applied across the bottom of a Whatman No. 1 paper and developed ascendingly using solvent a (Table 1). The chromatograms were air dried and the separated bands cut out and eluted from the paper. Anthocyanins were eluted with .01% HCl in methanol and the flavones and flavonols were eluted in 70% ethanol. These purified pigments were

concentrated by evaporation between 30° C and 40° C on a rotary vacuum evaporator.

The concentrated purified pigments were spotted on Whatman No. 1 filter paper and developed using solvents a, b, c, and d (Table 1) for anthocyanins and solvents a, b, c, f, j, and k (Table 1) for flavones and flavanols. The chromatograms were air dried and viewed in both visible and ultraviolet light before and after exposure to ammonia fumes. The spots were marked, changes noted, and R_f values calculated.

A Beckman model DB Spectrophotometer with a synchronized log recorder was used to measure and record the absorption spectra of the purified pigments. Spectral absorption curves were determined before and after the addition of 6 drops of an $AlCl_3$ solution, then after the addition of 3 drops of HCl solution to the cuvette containing the pigment and $AlCl_3$ (Mabry et al., 1970). The $AlCl_3$ and HCl may or may not cause shifts in the spectral absorption maxima which give information about the structure of the pigments.

Both the crude and purified pigments were hydrolyzed to gain information about the aglycones and the sugars that were attached to the anthocyanidin. Two methods of hydrolysis were tried. In one, equal amounts of pigment solution and 2N HCl were placed in a 25 ml Erlenmeyer flask covered with a glass marble and boiled directly on a hot plate for thirty minutes. In the other method equal amounts of pigment

and 2N HCl were placed in a 25 ml Erlenmeyer flask in a boiling water bath and were refluxed using a cold finger for 45 minutes. Samples were removed every five minutes to determine a stepwise degradation of the anthocyanins. In both methods when hydrolysis was finished the aglycones were separated from the other hydrolysis products with iso-amyl alcohol and were spotted on Whatman No. 1 paper and developed using solvents a, e, and f (Table 1). The chromatograms were air dried and the R_f values calculated (Table 2). The aqueous portion containing the sugars were spotted on Whatman No. 1 paper. Glucose and rhamnose were spotted as known sugars. The chromatograms were developed using solvents a, g, and h (Table 1). The chromatograms were air dried, sprayed with p-anisidine reagent, and placed in a 90° C oven for two to three minutes. After the color reaction had occurred, the sugar spots were marked, their R_f values calculated, and compared to the known spots.

RESULTS

Figure 2 contains drawings of the two dimensional chromatograms of the crude extracts of C. rapunculoides and C. americana. These show the pattern of the spots after developing in BAW for the first direction and HoAcHCl for the second dimension. Spots 1 and 2 were medium pink and turned blue when fumed with ammonia vapors. Spot 3 did not appear until after fuming with ammonia. It was invisible in

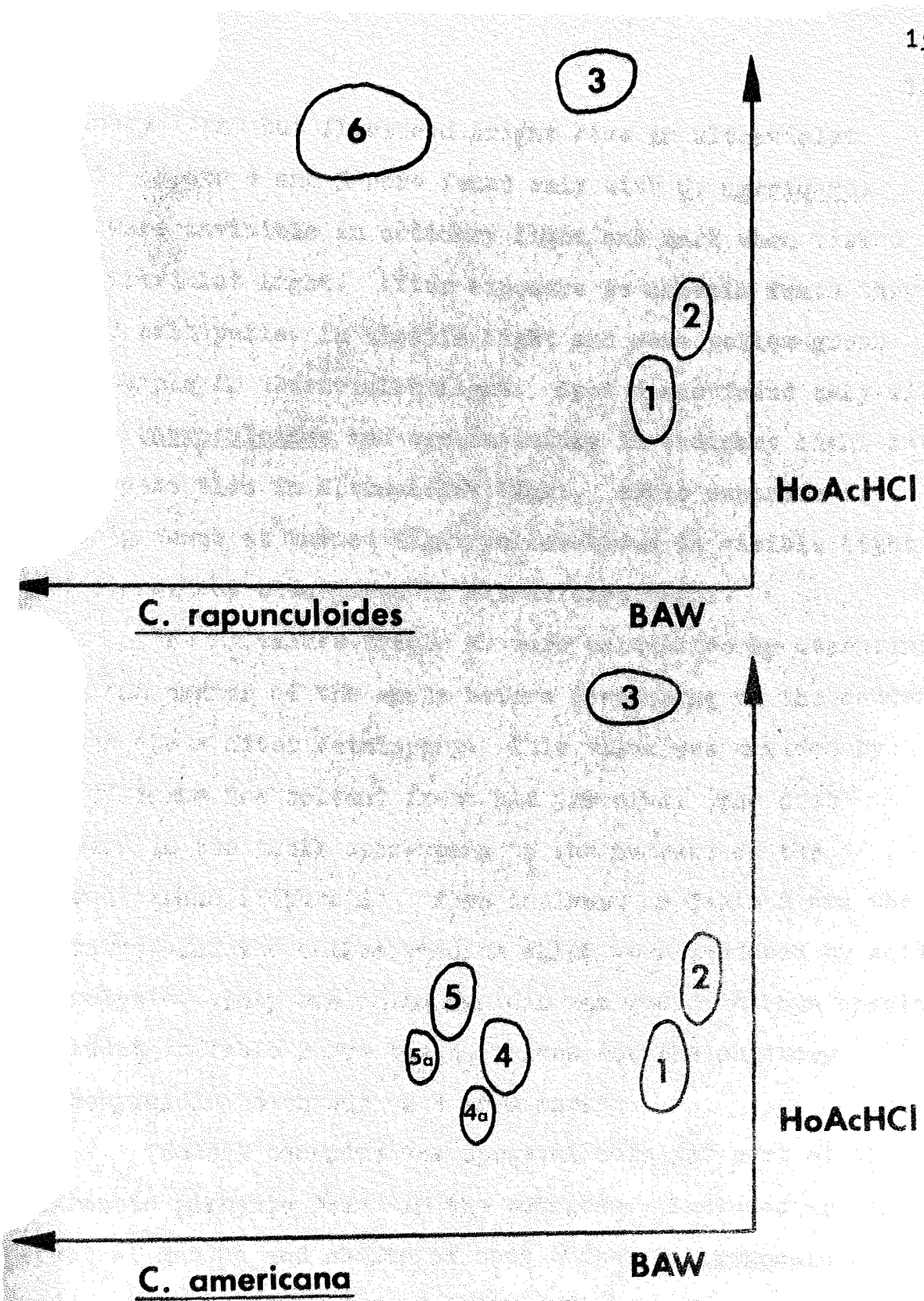


Figure 2. Chromatographic patterns showing similarities of C. rapunculoides and C. americana.

ordinary light but floresced bright blue in ultraviolet light. Spots 4 and 5 were found only with C. americana. They were invisible in ordinary light and dark when viewed in ultraviolet light. After exposure to ammonia fumes they turned pale yellow in visible light and were yellow-green with purple in ultraviolet light. Spot 6 was found only with the C. rapunculoides and was invisible in ordinary light but florescent blue in ultraviolet light. After exposure to ammonia fumes it turned light yellow-brown in visible light and florescent blue-green in ultraviolet light.

The R_f values (Table 2) were calculated by measuring from the center of the spots before developing to the center of the spots after developing. This value was divided by the distance the solvent front had traveled. The spot numbers in the table correspond to the numbers on the chromatograms (Figure 2). Also included in Table 2 are the R_f values for the anthocyanidins which were produced by acid hydrolysis. Only one anthocyanidin was found in both species. Included in Table 2 are the R_f values for the chickory anthocyanidin which was used as a marker.

Table 3 contains the spectral data for part of the flavonoid pigments found in the extracts. Included are the spectral maxima and shifts of spot 6 from C. rapunculoides and spots 4 and 5 of C. americana. There were no spectral shifts due to the addition of HCl. The anthocyanins showed a spectral maxima around 542 nm.

Table 2. R_f values of Pigments of C. rapunculoides and C. americana

Pigment	BAW	BuHCl	1%HCl	HoAcHCl	For.*	15%HoAc	Form.*	H_2O	TBA
C. rapunculoides									
Spot 1	.07	.04	.19	.45					
Spot 2	.15	.10	.07	.27					
Spot 6	.63		.49	.73	.84	.71			.70
Anthocyanidin	.40				.33		.19		
C. americana									
Spot 1	.09	.03	.17	.39					
Spot 2	.12	.4	.25	.41					
Spot 4	.24			.31					
Spot 5	.42			.45	.85	.34		.08	.86
Anthocyanidin	.43						.15		
Chickory									
Anthocyanidin	.43						.15		

*For. is Forrestal and Form. is Formic

Table 3. Spectral Data of Campanula Pigments

Pigment	Spectral Maxima 70% EtOH	with $AlCl_3$
C. rapunculoides		
Spot 6	325, 298sh, 235	same
C. americana		
Spot 4	350, 268sh, 255	390, 346, 298sh, 275
Spot 5	335, 265	380, 340, 300, 275
	Absolute MeOH	with $AlCl_3$
C. rapunculoides		
Spot 6	323, 290sh, 248sh	332-292, 252sh
C. americana		
Spot 4	330, 283sh, 270sh, 225sh	390, 343, 302sh, 275
Spot 5	342, 268, 256sh	388, 347, 298, 275

sh is for shoulder or inflection.

The sugar fraction produced by acid hydrolysis of the crude extracts were chromatographed in three solvent systems along with glucose and rhamnose as knowns. Glucose had R_f values of .18 in BAW, .19 in BEW, and .11 in EaPyW; rhamnose had values of .48 in BAW, .43 in BEW, and .66 in EaPyW. C. rapunculoides had two p-anisidine positive spots which showed up after hydrolysis. Their R_f values were .94 and

.32 in BAW, .90 and .23 in BEW, and .11 and .04 in EaPyW. C. americana also produced two sugars through hydrolysis. Their R_f values were .96 and .34 in BAW, .91 and .22 in BEW, and only one was visible in EaPyW with a value of .04. These values are given in Table 4.

Table 4. R_f values of p-anisidine positive spots after hydrolysis and known sugars.

Sugar	BAW	BEW	EaPyW
Glucose	.18	.19	.11
Rhamnose	.48	.43	.66
C. rapunculoides	.94	.90	.11
	.32	.23	.04
C. americana	.96	.91	not visible
	.34	.22	.04

DISCUSSION

The two dimensional chromatograms (Figure 2) of C. rapunculoides and C. americana show that they probably share two anthocyanins (spots 1 and 2) and one other flavonoid (spot 3). It is obvious that C. rapunculoides contains a blue florescing flavonoid that C. americana does not have (spot 6) and that C. americana has two flavonoid pigments (spots 4 and 5) that are not found in C. rapunculoides. In

part of the chromatograms there were sometimes two more flavonoid spots (4a and 5a) just below and to the left of spots 4 and 5, but these were very faint and no data was recorded for them.

The R_f values for the anthocyanidin of both C. rapunculoides and C. americana were very close to each other. They correspond closely to those reported by Harborne (1967) for delphinidin. They also were near those found for chickory which was chromatographed with them as a known delphinidin aglycone. Thus it appears that both C. rapunculoides and C. americana contain glycosides of delphinidin as their anthocyanins. The data suggest that they are the same in the two species. Their movements on the chromatograms always corresponded as well as their color in visible and ultraviolet light. Both had spectral maxima around 542 nm. The specific identity of the anthocyanins with their sugar attachments was not determined. It was thought perhaps that spot 2 might be delphinidin-3,5-diglucoside. The R_f values found for the anthocyanin of spot 2 correspond closely to those reported by Harborne (1967). However, identification of an anthocyanin must be based on sugar identification as well as R_f values and spectral maxima. The differences in movement of the anthocyanin in BAW is, in part, due to the sugar residues attached to the aglycone. Therefore, a slower moving anthocyanidin ought to have a heavier sugar residue attached. The data of the acid

hydrolysis and chromatography of the sugars was not sufficient to identify them and their position of attachment on the anthocyanidin. The data did suggest that both aglycones had the same sugar attachments. Since the R_f values of 0.12 and 0.15 are not high enough for the glucose-3,5-diglycoside, it may have a disaccharide attached at one of these two positions. This could be determined with controlled hydrolysis and study of the intermediate products.

No attempt was made to identify spot 3. Both species contain this pigment and it consistantly appeared in ultraviolet light after ammonia fuming. It probably is a 7,8-dihydroxyflavanone. Harborne (1959) stated they are different than other flavones in that they floresce bright blue on paper when fumed with ammonia in ultraviolet light.

Spots 4 and 5 were found only in C. americana. Time did not permit extensive hydrolysis work with these flavonoids. Thus the sugar attachments were not determined. From the R_f values and spectral curves produced, spot 4 appeared to be a glycoside of the flavone luteolin when compared with data in Harborne (1967), Geissman (1962), and Mabry et al. (1970). Spot 5 corresponded very closely with all literature to vitexin. Vitexin is a glycoflavone which has a glucose molecule attached by a carbon-carbon bond to the flavone nucleus in the 8-position (Harborne, 1967).

Spot 6 was found in abundance in C. rapunculoides but not in C. americana. The data for this spot was not sufficient

to positively identify it. Its color reactions seem to indicate that it is either an isoflavone or flavanone. The spectral curve is of the general shape of those given in Mabry et al. (1970) for isoflavones and flavanones but does not correspond to any in its spectral maxima. Harborne (1967) reported that flavanones have ultraviolet absorption maxima in a region of the spectrum where impurities can seriously interfere with their characteristic curves so this may account for the spectral differences.

Harborne (1963) reported several R_f values for delphinidin-3,5-diglucoside found in one species of Campanula. Only the R_f values were published and the details of the work were not given. No other work with Campanula has been reported. Wilson (1968) found delphinidin-3,5-diglucoside in a species of Lobelia. The relations between Campanula and Lobelia have not been settled. As previously reported some taxonomists include them in the same family and some in different families or subfamilies. The data found in this study confirms that reported by Harborne (1963). The fact that these two species of Campanula contain three pigments in common verifies their relationship. The fact that they do not share at least three other pigments shows they are not the same species. Since Campanula and Lobelia do share at least one pigment perhaps the classification should have both in the same family. More work with both species needs to be done to determine this.

SUMMARY

The purpose of this study was to identify and compare the flavonoid pigments in the flowers of Campanula rapunculoides and Campanula americana. Several pigments were tentatively identified and all were compared by chromatography.

Crude extracts of the flavonoids were compared and purified by filter paper chromatography. The spots were observed in visible and ultraviolet light both before and after exposure to ammonia fumes. R_f values were calculated for the various pigments and the pigments were examined with a spectrophotometer to determine their maximum absorption region. The pigments were hydrolyzed to separate the aglycones from the sugars and the products were chromatographed.

This study showed that C. rapunculoides and C. americana share two glycosides of delphinidin and probably one dihydroxyflavone. It was also shown that these two species differ in that they have different flavonoids. Vitexin and luteolin were found in the native species C. americana, while the C. rapunculoides differs in not having these compounds, but rather an as yet unidentified, blue florescing flavonoid.

This investigation suggests additional research is needed. Further work is needed with Campanula species to isolate and identify the sugars from the acid hydrolysis of the pigments. A controlled hydrolysis of the pigments and a study of the intermediate products should be done. Other

species of Campanula need to be examined to see if any pigments are shared with C. americana or C. rapunculoides. As to the problem of the position of Campanula and Lobelia in the family Campanulaceae, the aglycone delphinidin would indicate some relationship with Lobelia. However, more work needs to be done to determine the specific chemical relationship of Campanula to Lobelia.

LITERATURE CITED

Abe, Ykihide, and Kozo Hayashi. 1956. Further studies on paper chromatography of anthocyanins involving an examination of glycoside types by partial hydrolysis. Bot. Mag. (Tokyo) 69(822):577-585.

Alston, R. E., B. L. Turner, R. N. Lester, and D. Horne. 1962. Chromatographic validation of 2 morphologically similar hybrids of different origin. Sci. 137(3535): 1048-1050.

Arditti, Joseph. 1969. Floral anthocyanins in species and hybrids of Broughtonia, Brassavola, and Cattleyopsis (Orchidaceae). Amer. J. Bot. 56(1):59-68.

Arditti, J., and A. Dunn, ed. 1969. Experimental physiology. Holt, Rinehart, & Winston, New York. 174 pp.

Bate-Smith, E. C. 1948. Paper chromatography of anthocyanins and related substances in petal extracts. Nature 161: 835-838.

Bate-Smith, E. C. 1963. Usefulness of chemistry in plant taxonomy as illustrated by the flavonoid constituents. Pages 123-132 in T. Swain, Chemical plant taxonomy. Academic Press, New York.

Bell, C. Ritchie. 1967. Plant variation and classification. Wadsworth Publ. Co., Inc., Belmont, Calif. 131 pp.

Brown, G. B., J. R. Deakin, and M. B. Wood. 1969. Identification of Cucumis species by paper chromatography of flavonoids. J. Amer. Soc. Hort. Sci. 94(3):231-234.

Clevenger, S. 1964. Flower pigments. Sci. Amer. 210(6):85-93.

Conrad, H. S. 1948. Plants of Iowa, 6th Ed. Swift & Co., Chicago, Ill. 86 pp.

Conrad, H. S. 1951. Plants of Iowa, 7th Ed. Swift & Co., Chicago, Ill. 90 pp.

Deam, Charles C. 1940. Flora of Indiana. Dept. of Cons., Div. of Forestry, Indianapolis, Ind. 1166 pp.

Dedio, W., P. J. Koltsikes, and E. N. Lorter. 1969. The anthocyanins of Secale cereale. Phytochem. 8(12):2351-2352.

Durkee, A. B., and Jeffery B. Harborne. 1973. Flavonol glycosides in Brassica and Sinopis. Phytochem. 12(5): 1085-1089.

Fahselt, Dianne, and Marion Ownby. 1968. Chromatographic comparison of Dicentra species and hybrids. Amer. J. Bot. 55(3):334-345.

Francis, F. J. 1967. Criteria for identification of anthocyanins. Hort. Science 2(4):170-171.

Gage, T. B., C. D. Douglas, and S. H. Wender. 1951. Identification of flavonoid compounds by filter paper chromatography. Anal. Chem. 23(11):1582-1585.

Geissman, T. T. 1962. The chemistry of flavonoid compounds. The MacMillan Co., New York. 666 pp.

Gleason, Henry A. 1968. The new Britton and Brown illustrated flora, Vol. 3. Hafner Publishing Co., Inc., New York. 551 pp.

Gray, Asa. 1950. Gray's manual of botany, 8th Ed. American Book Co., New York. 1632 pp.

Gripenberg, Jarl. 1962. Flavones. Pages 406-440 in T. A. Geissman, The chemistry of flavonoid compounds. The MacMillan Co., New York.

Gupta, S. E. 1968. Thin layer chromatographic separation of anthocyanins and anthocyanidins in Medicaga (Papilionaceae). J. Chromatog. 36(1):115-119.

Halery, A. H., and S. Asen. 1959. Identification of the anthocyanins in petals of tulip varieties Smiling Queen and Pride of Harlem. Plant Phys. 34(5):494-499.

Harborne, J. B. 1958a. The chromatographic identification of anthocyanin pigments. J. Chromatog. 1:473-488.

Harborne, J. B. 1958b. Spectral methods of characterizing anthocyanins. Biochem. J. 70(1):22-28.

Harborne, J. B. 1959. The chromatography of the flavonoid pigments. J. Chromatog. 2:581-604.

Harborne, J. B. 1963. Distribution of anthocyanins in higher plants. Page 472 in T. Swain, Chemical plant taxonomy. Academic Press, New York.

Harborne, J. B. 1967. Comparative biochemistry of the flavonoids. Academic Press, London. 344 pp.

Hattori, Shizuo. 1962. Glycosides of flavones and flavonols. Pages 317-352 in T. A. Geissman, The chemistry of flavonoid compounds. The MacMillan Co., New York.

Hayashi, Kozo. 1962. The anthocyanins. Pages 248-285 in T. A. Geissman, The chemistry of flavonoid compounds. The MacMillan Co., New York.

Jurd, Leonard. 1962. Spectral properties of flavonoid compounds. Pages 107-155 in T. A. Geissman, The chemistry of flavonoid compounds. The Macmillan Co., New York.

Mabry, T. J., K. R. Markham, and M. B. Thomas. 1970. The systematic identification of flavonoids. Springer-Verlag, New York. 343 pp.

McClure, Jeny W., and Ralph, E. Alston. 1966. A chemotaxonomic study of Lemnaceae. Amer. J. Bot. 53(9): 849-860.

Muszynski, Stanislaw. 1964. A survey of anthocyanidins in petunia. Physiol. Plant 17(4):975-979.

Noriyuki, Ihikura. 1971. Anthocyanin patterns in the genera of Ilex and Euonymus. Phyto. Chem. 10(10):2513-2517.

Pollock, H. Gail, Robert K. Vickery, Jr., and Kenneth G. Wilson. 1967. Flavonoid pigments in Mimulus cardinalis and its related species. I anthocyanins. Amer. J. Bot. 54(6):695-701.

Rendle, Alfred Barton. 1925. The classification of flowering plants. Cambridge Univ. Press, London. 614 pp.

Robinson, G. M., and R. Robinson. 1934. A survey of anthocyanins IV. Biochem. J. 28(5):1712.

Seikel, Margaret K. 1962. Chromatographic methods of separation, isolation and identification of flavonoid compounds. Pages 34-69 in T. A. Geissman, The chemistry of flavonoid compounds. The MacMillan Co., New York.

Smith, Ivor. 1969. Chromatographic and electrophoretic techniques, Vol. 1. Interscience Publishers, New York. 1060 pp.

Stewart, R. N., S. Asen, K. H. Norris, and D. R. Massie. 1969. Relation of flower color to optical density spectra of intact tissues and of anthocyanin extracts. Amer. J. Bot. 56(2):227-231.

Thompson, J. F., ed. 1959. Partition chromatography and its use in the plant sciences. Botan. Rev. 25(1):1-263.

Torres, B. L., and D. A. Levin. 1964. A chromatographic study of cespitace Zinnias. Amer. J. Bot. 51(10):639-643.

Valdes, B. 1970. Flavonoid pigments in flowers and leaves of the genus Linaria (Schrophulariaceae). Phytochem. 9(6):1253-1260.

Wilson, S. Lane. 1968. Anthocyanin pigments in the genus Lobelia: I. Pigments in Lobelia cardinalis L. and L. siphilitica L. Iowa Academy of Sci. 75:65-68.